Regionalization of the stretch reflex in the human vastus medialis

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KEY POINTS SUMMARY:

- Regionalization of the stretch reflex, i.e.: the notion that the activation of 1a afferents from a muscle region influences only the activation of motor units in the same region, has been demonstrated previously in animals but not in humans.
- Mechanical stretches applied to regions of vastus medialis as close as 10 mm apart resulted in recruitment of motor units localized topographically with respect to the location of the mechanical stretch.
- Stretch reflexes are regionalized in the human vastus medialis.
- The human spinal cord has the neuromuscular circuitry to preferentially activate motoneurones innervating muscle fibres located in different regions of the vastus medialis.

ABSTRACT:

The localization of motor unit territories provides an anatomical basis to suggest that the CNS may have more independence in motor unit recruitment and control strategies than what was previously thought. In this study, we investigated whether the human spinal cord has the neuromuscular circuitry to independently activate motor units located in different regions of the vastus medialis.

Mechanical taps were applied to multiple locations in the vastus medialis (VM) in nine healthy individuals. Regional responses within the muscle were observed using a grid of 5×13 surface EMG electrodes. The EMG amplitude was quantified for each channel, and a cluster of channels showing the largest activation was identified. The spatial location of the EMG response was quantified as the position of the channels in the cluster. In a subset of 3 participants, intramuscular recordings were performed simultaneously with the surface EMG recordings.

Mechanical taps resulted in localized, discrete responses for each participant. The spatial location of the elicited responses was dependent on the location of the tap ($P < 0.001$). Recordings with intramuscular electrodes confirmed the regional activation of the VM for different tap locations.

Selective stimulation of 1a afferents localized in a region of the VM results in reflex recruitment of motor units in the same region. These findings suggest that the human spinal cord has the neuromuscular circuitry to modulate spatially the motoneuronal output to vastus medialis regions, which is a neuroanatomical prerequisite for regional activation.

KEYWORDS: Motor unit; stretch reflex; EMG; quadriceps; spinal cord; neuromechanics

ABBREVIATIONS LIST: CNS: Central Nervous System; VM: Vastus Medialis; HDsEMG: High-Density surface Electromyography; EMG: electromyographic; MVC: Maximal Voluntary Contraction; CoV: Coefficient of Variation.

INTRODUCTION:

Motor units, consisting of the motoneurone and the muscle fibres it innervates, provide the conduit for the central nervous system (CNS) to convert neural signals into forces. It has been known for years that motoneurone soma size is a main determinant of motor unit recruitment order, with smaller motoneurones activated before larger ones (Henneman, 1957), and that motor units within individual muscles and across synergistic muscles share a common command from the CNS (De Luca & Erim, 1994; Laine *et al.*, 2015). However, the existence of motor units with localized territories in humans suggests that the CNS may have more independence in motor unit recruitment and control strategies than previously thought. For example, most (Buchtal *et al.*, 1959; Gootzen *et al.*, 1992; Vieira *et al.*, 2011; Gallina & Vieira, 2015), 70% (Harris *et al.*, 2005) or up to half (Héroux *et al.*, 2015) of the motor units in human muscles have localized territories, meaning that motoneurones innervate muscle fibres clustered in limited muscle regions. While the functional implication of this anatomical structure is currently unknown, it constitutes a basis for the CNS to distribute neural inputs to motoneurones based on the location of the muscle fibres they innervate. This was suggested as a possible mechanism for the CNS to take advantage of heterogeneous muscle architecture (Windhorst *et al.*, 1989; Vieira *et al.*, 2011), and for changes in motor control strategies in the presence of pain (Tucker *et al.*, 2009); yet, there is currently no evidence for the existence of such selective motor unit recruitment in humans.

The regionalization of the stretch reflex requires that motor units located in different muscle regions can be selectively recruited by the human spinal cord. Due to its complex architecture, the vastus medialis (VM) offers a good opportunity to study the regionalization of the stretch reflex in humans. The VM has a distributed insertion along 40-60% of the medial side of the patella (Holt *et al.*, 2008) and on the common quadriceps tendon (Smith *et al.*, 2009). Proximal-to-distal differences were observed in the orientation of VM muscle fibres (Smith *et al.*, 2009; Gallina & Vieira, 2015), pennation angle (Blazevich *et*

al., 2006; O'Brien *et al.*, 2010), thickness (Blazevich et al. 2006; O'Brien et al. 2010) and fibre length (O'Brien *et al.*, 2010). Because of these regional differences in muscle architecture and insertion, muscles fibres located in the proximal or distal VM exert forces directed proximally or medially on the patella (Lin *et al.*, 2004) despite the absence of clear anatomical compartmentalization (Smith *et al.*, 2009). Also, motoneurones supplying the human VM innervate muscle fibres confined to limited regions within the muscle (Gootzen *et al.*, 1992; Gallina & Vieira, 2015), which is a neuro-anatomical prerequisite for regional activation. Regionalization of the stretch reflex may be a mechanism the CNS uses to coordinate the activation of VM regions with different mechanical properties.

In this study, we investigated whether motoneurones innervating muscle fibres located in different regions of the human VM can be independently recruited at the spinal level. Regional activation of 1a afferents localized in different VM regions was produced through muscle taps and the spatial location of the recruited motor units was investigated using a grid of surface electromyographic electrodes (Vieira *et al.*, 2011) and confirmed with intramuscular fine-wire recordings. It was hypothesized that stretch reflexes within the VM are localized and vary systematically with the location of the tap. If localized stretch of muscle fibres results in regional activation within the muscle, this would demonstrate that motor units located in different regions can be independently recruited at the spinal level.

METHODS:

Participants

The screening process consisted of applying taps along the VM insertion on the patella and visually assessing whether muscle twitches were elicited. Reflex contractions were observed in all screened individuals but some reported discomfort due to the large amount of force needed to elicit the reflex and were not further tested. Nine individuals participated in this study (1 female; 24 - 53 years old). All

participants signed a written informed consent form. The study conformed to the standards set by the latest revision of the *Declaration of Helsinki* and was approved by the University of British Columbia Clinical Research Ethics Board.

High-Density surface Electromyography

Placement of the High-Density surface Electromyography (HDsEMG) grid was guided by anatomical references. The medial and lateral boundaries of the VM were identified with ultrasound imaging (LogicScan 64 LT-1T, Telemed, Vilnius, Lithuania) and were marked on the skin. The innervation zone of VM was located using a linear electrode array (16 silver bar electrodes, 10mm inter electrode distance, OTBioelettronica, Torino, Italy) moved over different regions of the muscle along muscle fibres while the participants maintained a low-force isometric knee extension contraction. The innervation zone, identified by the bi-directional propagation of the bipolar action potentials observed in consecutive channels, was marked on the skin. Similar to a previous study (Gallina & Vieira, 2015), the innervation zone of fibre groups was found to be oriented diagonally across the VM (fig. 1). The HDsEMG grid (semi-disposable adhesive matrix; OTBioelettronica, Torino, Italy) consisted of 64 electrodes arranged in 5 columns and 13 rows (an electrode missing in one of the corners), spaced by 8 mm with a total area covered by the electrodes of 3072 mm^2 (96×32 mm). The grid was placed proximally to the innervation zone with the long axis of the grid (columns) parallel to it. The distal column of electrodes (column 1) was placed approximately 5 mm from the estimated location of the innervation zone and the medial row of electrodes (row 1) was close to the medial border of the VM muscle (fig. 1). Bi-adhesive foam held the grid in place and conductive paste (Ten20, Weaver and Co., Aurora, CO, USA) ensured good electrical contact between the skin and electrodes. Two surface electrodes (20×35 mm; conductive hydrogel; Kendall, Covidien, Mansfield, MA, USA) were placed on the medial side of the knee as reference electrodes.

Intramuscular recordings

As differences in motor unit territory estimates in the medial gastrocnemius were recently observed when assessed using HDsEMG versus intramuscular recordings (Vieira *et al.*, 2011; Héroux *et al.*, 2015), intramuscular multiunit electromyographic (EMG) signals were recorded in three participants together with HDsEMG. Custom-made electrodes consisted of two 0.05 mm insulated stainless steel wires (California FineWire, Grover Beach, CA, USA) wound together and inserted via a 1.75 inch 25 gauge hypodermic needle (EXEL International Medical Products, St Petersburg, FL, USA). The threaded wires were folded back to create one 4 mm barb and one 10 mm barb, with the insulation removed from the distal 5 mm (longer barb) or 2 mm (shorter barb) to form the recording sites. The large exposed areas of the wires were chosen in order to favour multi-unit EMG recordings. Three wire electrodes were inserted under ultrasound guidance at a ~10 mm depth along the 3rd column of HDsEMG electrodes at rows 3, 7 and 11. A surface electrode was placed over the lateral femoral epicondyle and served as the ground for fine-wire electrode recordings.

Protocol

Participants sat in the chair of a Biodex dynamometer (System 4 Pro, Biodex Medical Systems, Shirley, NY, USA) with their lower leg strapped to the knee attachment at 80 degrees knee flexion. Taps were manually applied using a custom-made hammer with a load cell embedded (Force-Displacement Transducer FT 10, Grass Instrument Co., Quincy, Mass, USA). The head of the hammer was a plastic cone with a rounded tip (5 mm diameter). Taps were applied orthogonally to the skin over the VM muscle fibres by the same investigator in all participants while monitoring the tap force and the EMG response on a computer screen. Taps were first applied close to the distal insertion of the most distal fibres of the VM, and then moving proximally following the patellar edge until a clear response was observed (L1, fig. 1). The other locations (5 locations maximum) were identified by applying taps progressively more proximally along the edge of the patella in steps of 10 mm until no responses could be observed (fig. 1). For each location, taps were applied starting at the edge of the patella, and then moving away from the patella along the muscle

fibres. The location that provided the largest EMG responses while minimizing artefacts was marked on the skin (fig. 1). Thirty taps were applied to each location with varying input force to obtain a range of reflex response amplitudes; surface EMG activity was carefully monitored online to ensure that the VM was at rest when taps were applied. Following muscle taps, participants who took part in the validation with intramuscular EMG recordings were asked to perform three isometric maximal voluntary contractions (MVC) with verbal encouragement. Tap locations were marked and measured on a coordinate system referenced to the centre of the patella.

Surface EMG signals were collected in monopolar configuration using an HDsEMG amplifier (128-channel EMG-USB; OTBioelettronica, Torino, Italy). Signals were amplified (×500-1000), filtered (band-pass 10-750 Hz) and digitized at 2048 Hz using a 12 bit A/D converter. Differential fine-wire EMG signals were filtered (band-pass 30–6000 Hz; NL 134 and NL 844, Digitimer, Garden City UK), amplified (×1000; NL 820 A and NL 844, Digitimer, Garden City UK) and then A/D converted at 20 kHz (Power 1401 with Spike2 software, Cambridge Electronic Design, Cambridge, UK). The force signal was amplified (×100), low-pass filtered (10 KHz) and simultaneously digitized by the two acquisition systems used for EMG recordings; the force signal collected at 20kHz was used for analysis.

Data analysis

All data analysis was performed in Matlab R2013b (The MathWorks, Inc., Natick, MA, USA). EMG signals were band-pass filtered (dual-pass Butterworth, 4^{th} order for each direction; surface: 20-400 Hz; intramuscular: 300-2000 Hz) before analyses. Tendon taps were identified using the force measured with the force transducer placed in the hammer. The timing of each tap was identified as the first data point after which the force signal reached 5% of the peak force amplitude (fig. 2). Epochs from 50 ms before to 450 ms after each tap were analyzed. Surface EMG channels showing artefacts or predominantly power line interference, as determined by visual inspection (less than 10% of the channels; range: 0-7 channels), were replaced by the linear interpolation of the four adjacent channels. The onset of the response and

the occurrence of action potential propagation along the rows of the electrode grids were used to distinguish the presence of a spinal reflex from mechanical artefact. Only taps that resulted in clear negative peaks delayed by 1-3 ms in channels progressively more proximal along the VM fibres (further away from the neuromuscular junction) were included in the analysis (fig. 2). Because mechanical taps were applied in a range of forces (see below), no muscle activation in response to the tap was observed in 22% of the trials across all locations. These trials were excluded from all analyses.

For each channel, the magnitude of the surface EMG response was calculated as the amplitude of the largest negative peak occurring 15-45 ms after the tap. Artefacts due to the tap could sometimes be observed superimposed on the EMG response in columns 1 and 2 and these columns were excluded from the analysis for all participants. An example of surface EMG signals can be observed in fig. 3 top rows. For each of the taps where an EMG response was observed, the amplitude values of columns 3-5 of each row were averaged obtaining an array of 13 values (an example is shown in fig. 1 above the EMG grid). Thus, in each single tap location, up to 30 arrays of amplitude values representing the distribution of the reflex response along the columns of the grid were established.

Figure 4 shows the amplitude range of the responses to the manually-evoked taps from the intramuscular recordings of a representative subject. A consistent spatial localization of the response is observed despite the difference in amplitude. As muscle thickness (Blazevich *et al.*, 2006) and the amount of skin/fat tissues (Botter *et al.*, 2011) changes across the VM, applying the same input force across the tap locations does not necessarily ensure similar muscle spindle activation. To ensure that the localization of the stretch reflex across tap locations was not influenced by input force or amplitude of the EMG responses, three separate analyses were conducted: all trials (all mechanical taps), trials matched for input (force-matched) or trials matched for output (EMG amplitude-matched). Force or EMG amplitude matching across different tap locations was done separately. For the force-matched analysis, the largest five taps in each location were selected and the input force values were averaged. The lowest of the average values among

199 locations was chosen as reference. For each location, the 5 trials with input force closest to this reference
200 value were selected and included in the analysis. For the EMG amplitude-matching analysis, selection of
201 the trials was done following the same procedure but trials were matched for amplitude of the EMG
202 responses instead of force input. For the force-matched and amplitude-matched responses, the
203 coefficient of variation (CoV) was calculated for each participant as the standard deviation divided by
204 mean across tap locations and expressed as a percentage. This index was used to describe the variability
205 of force and EMG amplitude across tap locations to verify that the matching was effective.
206 In all analyses, the amplitude distributions of the selected trials (5 out of 30 for force-matched and
207 amplitude-matched analyses) or of all trials were averaged for each location, resulting in one array of 13
208 amplitude values per tap location. Position, amplitude, size and latency of the responses were identified
209 for each averaged distribution as follows: a cluster of channels with amplitude larger than 40% of the
210 maximal value of the 13 channels were identified (threshold determination detailed below; fig. 5), and: i)
211 the size of the active region within the VM was calculated as the number of channels included in the
212 cluster; ii) the localization of the EMG response was described as the barycentre of the channels,
213 calculated as

214 $$\text{barycentre} = \frac{\sum ARV_{ch} POS_{ch}}{\sum ARV_{ch}}$$

215 with *ch* being each channel in the cluster, *ARV* being their Average Rectified Value (measure of amplitude),
216 *POS* being their position in the array. The channels in the cluster were used to estimate the latency of the
217 response, calculated as the average timing between the onset of the tap and the negative peak of each of
218 the channels.
219 For intramuscular recordings, the same taps analyzed in the all-trials surface EMG analysis for locations 1,
220 3 and 5 were included in the analysis. The amplitude of the response for each wire was calculated as the
221 root mean square value in the 10-50 ms window after tap onset. The amplitude of the baseline noise (root
222 mean square value of an epoch 10-50 ms before the tap) was subtracted from the amplitude of the

response. For each tap, the amplitude of the EMG response in each wire was expressed as a percentage of root mean square value measured during isometric maximal knee extension (maximal value of 50 ms epoch calculated with 45 ms overlapping windows). For each tap location, the normalized amplitude measured in each of the three intramuscular electrodes was averaged across the thirty taps resulting in a matrix of 3 participants × 3 tap locations × 3 fine-wire locations.

The 40% threshold for the surface EMG analysis was chosen based on the concurrent analysis of the surface and intramuscular EMGs in the subset of three experiments where both EMGs were collected. For each intramuscular recording location, the surface EMG amplitude distribution averaged over all trials was compared to the intramuscular recordings. As seen in fig. 3 (bottom rows) no EMG responses were observed in the wires other than the wire corresponding to the location of the mechanical tap. This indicated that the low level EMG activity registered by the surface electrodes located above the intramuscular wires with no EMG activity was not a response to the mechanical tap but was due to volume conduction or crosstalk. Therefore, a series of thresholds from 5 to 95% of the peak value of the surface EMG amplitude distribution were tested for each tap location (3 participants with 3 tap locations each). The lowest threshold that excluded surface EMG channels placed above the intramuscular EMG locations that exhibited no activity was selected for each location. The average threshold value across all 9 tap locations (40%; 38.8% rounded up to the closest 5%, range: 25-50%) was used to analyze all data from the HDsEMG. This threshold value is more conservative than the 70% used in other studies (Vieira *et al.*, 2010) and will lead to larger estimated regions of active muscle fibres.

Statistical Analysis

Statistical analyses were performed using SPSS v. 22 (IBM Inc., Armonk, NY, USA). When data were not normally distributed (Shapiro-Wilk test), non-parametric statistics were used. To verify that the input force or amplitude of the response were effectively matched in the corresponding analyses, Friedman

247 tests were run to assess the effect of *Tap location* on input force (force-matched) or EMG amplitude of
248 the response (amplitude-matched); the variability of input force and EMG amplitude values across
249 locations was also verified using the CoV. To investigate the regionalization of the stretch reflexes within
250 the VM, the number of channels in the cluster was used as a measure of size of the active area within the
251 VM and the barycentre of the channels in the cluster was used as a measure of spatial localization of the
252 active area within the VM. The effect of 'Tap location' on the number of channels in the cluster was tested
253 using the Friedman test. The effect of 'Tap location' on the barycentre of the channels in the cluster was
254 tested using ANOVAwith repeated measures, performed separately for force-matched, amplitude-
255 matched and all-trial analyses. Separate analyses were run to avoid violations of the assumption of
256 independent observations for the ANOVA test. As reflexes from locations 5 and 4 were not observed in
257 some participants, only locations 1, 2 and 3 were compared (additional locations are shown in fig. 6).
258 Post-hoc decompositions of main effects were performed using paired Student T-tests with Bonferroni
259 correction; for each pair of locations, effect sizes were calculated as:

260 $$d = \frac{mean}{SD}$$

261 where *mean* and *SD* are the mean and standard deviation of the difference between the groups. Results
262 from the validation with intramuscular electrodes are reported as the average across participants. Data
263 are reported as mean and standard deviation unless specified otherwise. The statistical significance was
264 set at $P < 0.05$.

265

RESULTS:

Localized muscle twitches could be visually observed in all participants (video: Muscle twitches in response to mechanical taps). Reflexes were observed in the surface EMG signals as a single burst of activity (fig. 3), with a mean latency of the largest negative peak of approximately 29 ms (force-matched: 29.2 ± 3.6 ms; amplitude-matched: 28.9 ± 3.6 ms; all-taps: 28.9 ± 3.4 ms). No medium- or long-latency EMG responses to the taps were observed. No muscle activation was observed before applying the mechanical taps (average rectified value calculated on a 100 ms window 50 ms before the taps, mean: 3.6±0.9 μV).

There was no difference in force or EMG response amplitude across tap locations for the trials selected based on these measures, respectively, confirming that the matching was effective (force-matched: $P = 0.89$, CoV = 3.4 ± 1.8% across participants, 25th-75th percentiles: 13.3 - 22.7 N; EMG amplitude-matched: $P = 0.36$; CoV = 9.6 ± 5.4%, 57 - 166 μV; N=5 taps in each location). The response to a tap always consisted in a single area of activity within the VM. The size of this active area spanned only few channels for all tap locations (fig. 5) with the median being 5 channels irrespective of the tap location for any analysis (all-trials: $P=0.14$, 25th–75th percentiles: 4.5–6; force-matched: $P = 0.07$, 5–6; amplitude-matched: $P = 0.08$, 4–6). All participants showed responses when taps were applied in the distal region of the muscle (locations 1-3 in fig. 1). Taps applied to locations 4 and 5 elicited EMG responses in 8 and 5 participants, respectively (fig. 6). The location of the tap influenced the localization of the EMG response on the grid (all-trials: $P < 0.001$, $F(2,16) = 45.5$; amplitude-matched: $P < 0.001$, $F(2,16) = 51.5$; force-matched: $P < 0.001$, $F(2,16) = 60.5$; Fig. 6). For all analyses, post-hoc testing revealed that each location was different from the other two (all $P < 0.01$; $t > 3.6$), resulting in large effect sizes ($d>1.2$). Taps applied more proximally along the patella resulted in more proximal responses within the VM than taps applied more distally. This localization of the response was confirmed by the intramuscular EMG recordings (fig. 3). Taps applied more proximally along the patella resulted in more proximal responses

within the VM than taps applied more distally. This localization of the response was confirmed by the intramuscular EMG recordings (Fig. 3). Taps applied to the distal location (location 1) resulted in larger EMG responses in the intramuscular wire placed distally in the VM (7.5% MVC distal; 0.7% MVC middle; 0.1% MVC proximal). Similar patterns of localized responses were observed for taps applied to the middle (0.1%MVC distal; 11.8% MVC middle; 0.4% MVC proximal) and proximal location (0.9%MVCdistal; 2.3%MVCmiddle; 9.1%MVC proximal). Responses for each participant are presented in Table 1.

DISCUSSION:

The regionalization of the stretch reflex observed in this study implies that the human spinal cord can independently recruit motoneurones innervating muscle fibres located in different regions within the VM. As regional recruitment was observed in response to the activation of 1a afferents localized in regions separated by only 10 mm, it follows that the human spinal cord has the circuitry to control motoneuronal output regionally based on motor unit location.

Mechanical taps applied to the VM muscle fibres and HDsEMG enabled the characterization of the spatial relation between regional stimulation of 1a afferents and location of the motor units recruited by the spinal cord. Mechanical taps were used to activate muscle spindles located in different regions of the VM. Although techniques such as the Hoffman reflex enable a fine control of the input and consistency across trials and conditions (McNeil *et al.*, 2013), mechanical taps can target muscle spindles located in different regions within large, flat muscles. HDsEMG was used to investigate the localization of the EMG response within the VM. As the surface EMG amplitude peaks above the active motor units and decreases with distance from the active muscle fibres (Roeleveld *et al.*, 1997), surface EMG amplitude distribution obtained with HDsEMG provides information on the position of the active motor units within a muscle (Roeleveld *et al.*, 1997; Vieira *et al.*, 2011; Gallina & Vieira, 2015; Gallina *et al.*, 2016). Spatial localization

was confirmed in a subset of participants using multiple intramuscular recordings, validating the findings of the HDsEMG as localized activation was observed using both HDsEMG and intramuscular electrodes. Using a threshold based on the intramuscular recordings, this study identified active areas spanning 5 channels of the grid (median value) in response to the mechanical taps. The 40% threshold we used is more conservative than the threshold value previously utilized to identify regional activation in simulated EMG signals (Vieira *et al.*, 2010). Similar spatial localization for different tap location was also observed when data were analyzed using the 70% threshold (analysis not reported), although the active muscle region was smaller (3 channels, median value). Regardless of the threshold used, the present results imply that the active VM region in response to mechanical taps is not larger than 5 channels. This value, however, may be an overestimation and further experimental validation is needed to determine the threshold that accurately defines the contracting muscle region. Regardless, there are similarities between the regional activation observed in the current and a previous study employing selective, intramuscular stimulation (Gallina *et al.*, 2016). Previous research on the relationship between surface EMG amplitude distribution and active fibres (Roeleveld *et al.*, 1997) and the results of the intramuscular recordings in this study strongly suggest that activation in response to the mechanical tap was regionalized within the vastus medialis.

Taps applied to muscle fibres in specific VM regions resulted in EMG reflex responses preferentially observed in some channels of the electrode grid and in a single intramuscular site. In addition, mechanical taps applied to muscle fibres in different VM locations resulted in EMG responses localized in different regions within the muscle. This indicates that the excitation of muscle spindles of a limited region of the muscle does not result in reflex activation of the whole muscle, but instead the reflex is confined to a specific region. Localized activation of the VM to mechanical taps was observed regardless of which taps were included in the analyses (force-matched, amplitude-matched, all-taps), strongly supporting the main results of this study. The localization of the stretch reflex implies regionalization at

three levels of the spinal circuitry: preferential response of 1a afferents located in different regions of the target muscle, specific connection of these afferents to motoneurones innervating the same muscle region in the spinal cord, and motoneurones innervating fibres confined in a region of the muscle (Windhorst *et al.*, 1989). Regional response of 1a afferents was demonstrated in animals (Cameron *et al.*, 1981) and humans (McKeon *et al.*, 1984), where mechanical stimuli applied to regions of a muscle were shown to result in discharges of 1a afferents from those regions only. Our results support previous observations suggesting that motoneurones innervate VM muscle fibres confined to limited regions of the muscle (Gootzen *et al.*, 1992; Gallina & Vieira, 2015). The localization of the stretch reflex was shown in cats (Cohen, 1953; Bilotto *et al.*, 1982; Eng & Hoffer, 1997) but not in humans (McKeon *et al.*, 1984). Differences in the results between our study and the one by McKeon and colleagues (McKeon *et al.*, 1984) may be related to differences in the architecture between the two muscles, e.g.: single long tendon vs. flat insertion along the patellar edge. To our knowledge, this is the first evidence for the regionalization of the stretch reflex in humans. Our results further reveal that this regionalization is distributed quite finely, as clearly separated responses could be observed for locations as close as 10 mm apart. Similarly to the observations for directional preference of motor unit activation in biceps brachii and deltoid (Herrmann & Flanders, 1998), our results indicate that stretch reflexes can be elicited continuously across the VM rather than clustering in anatomically-defined neuromuscular compartments (e.g.: VM longus or obliquus, (Smith *et al.*, 2009)).

The regionalization of the stretch reflex may potentially modulate the motor output of large, structurally complex muscles such as the VM. For instance, selective stretch reflexes may be useful in the case of perturbations that result in preferential stretch of a muscle region, such as sudden directional translation of the patella or tibio-femoral rotation which may occur especially in certain activities or sports. The current study indicates that the human spinal cord has the neuromuscular circuitry to modulate spatially the motoneuronal output to vastus medialis regions based on regional afferent

362 feedback. It has been suggested that regionalization of afferents and efferents may be used by the central
363 nervous system to shape patterns of activation in order to optimize muscle performance (Windhorst *et*
364 *al.*, 1989). The abundance and distribution of muscle spindles in human muscles was suggested to be
365 functionally useful to detect regional changes in length within the muscle and locally regulate the
366 motoneuronal output (Windhorst *et al.*, 1989). Indeed, while the synaptic input is largely shared across
367 motor units both within single muscles and between synergists (De Luca & Erim, 1994; Laine *et al.*, 2015),
368 the common drive between motor units tends to be lower in muscles with a higher density of spindles (De
369 Luca *et al.*, 2009). This suggests that afferent proprioceptive information from muscle spindles may
370 promote more independent motor unit firing patterns. The current study adds that the spinal cord has
371 the circuitry to spatially organize the 1a stretch response within a muscle. Furthermore, this study shows
372 that the human spinal cord has the neuromuscular circuitry to preferentially drive motor units localized
373 in different muscle regions. This constitutes a neuroanatomical substrate for reports of region-specific
374 motor unit recruitment (Herrmann & Flanders, 1998; Butler & Gandevia, 2008) and inhomogeneous
375 alteration of motor unit recruitment and firing rate in the condition of experimental pain (Tucker &
376 Hodges, 2009; Tucker *et al.*, 2009). Future studies should investigate whether regional activation of
377 afferents during voluntary contractions can alter motor unit recruitment strategies.

378 Overall, our results showed that mechanical stimulation of 1a afferents localized as close as 10
379 mm apart within the human VM resulted in regional recruitment of motor units whose location was
380 organized topographically with respect to the stimulus location. This indicates that the human spinal cord
381 has the neuromuscular circuitry to preferentially modulate the neural drive directed to motor units
382 residing in different muscle regions, which is a neuroanatomical prerequisite for regional activation of
383 skeletal muscles.

384

ADDITIONAL INFORMATION:

Competing Interests: The authors declare no competing interests.

Author contributions: Conception and design of the experiments: AG, JSB, SJG; Collection, assembly, analysis and interpretation of data: AG, TI, SJG; Drafting the article or revising it critically for important intellectual content: AG, JSB, TI, SJG. All authors approved the final version of the manuscript. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed. The experiments were run in the Neural Control of Force Production and Movement Laboratory, University of British Columbia, Canada (Dr. SJ Garland).

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TABLE:

Table 1: Intramuscular EMG activation (%Maximal Voluntary Electrical activation) in response to mechanical taps. Rows identify different tap locations (DISTAL – location 1; MIDDLE – location 3; PROXIMAL – location 5); columns represent the three wires placed distally (D), middle (M) and proximally (P). Each row depicts the EMG responses in the three muscle locations for the same mechanical stimulation. For each participant, the wire with expected largest response (gray) recorded amplitude higher than the other two muscle regions in the same row

	PARTICIPANT 1			**PARTICIPANT 2**			**PARTICIPANT 3**		
	D	**M**	**P**	**D**	**M**	**P**	**D**	**M**	**P**
DISTAL	3.7	2.0	0.2	1.0	0.0	0.0	17.8	0.1	0.0
MIDDLE	0.1	12.8	0.3	0.0	0.4	0.0	0.0	22.2	0.9
PROXIMAL	0.3	2.4	8.1	0.9	0.8	1.4	1.6	3.5	17.8

FIGURES CAPTIONS:

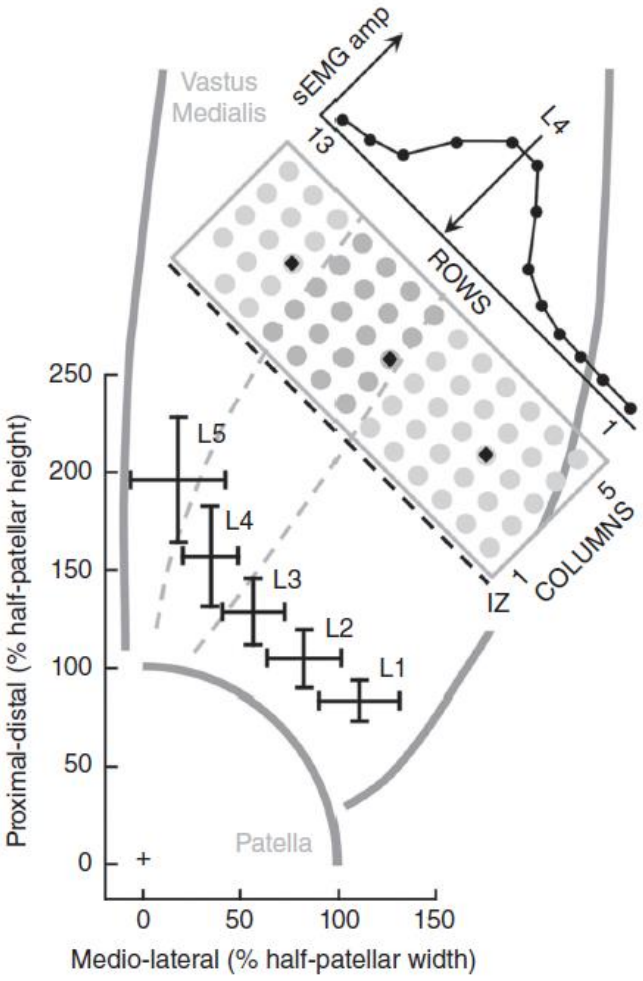
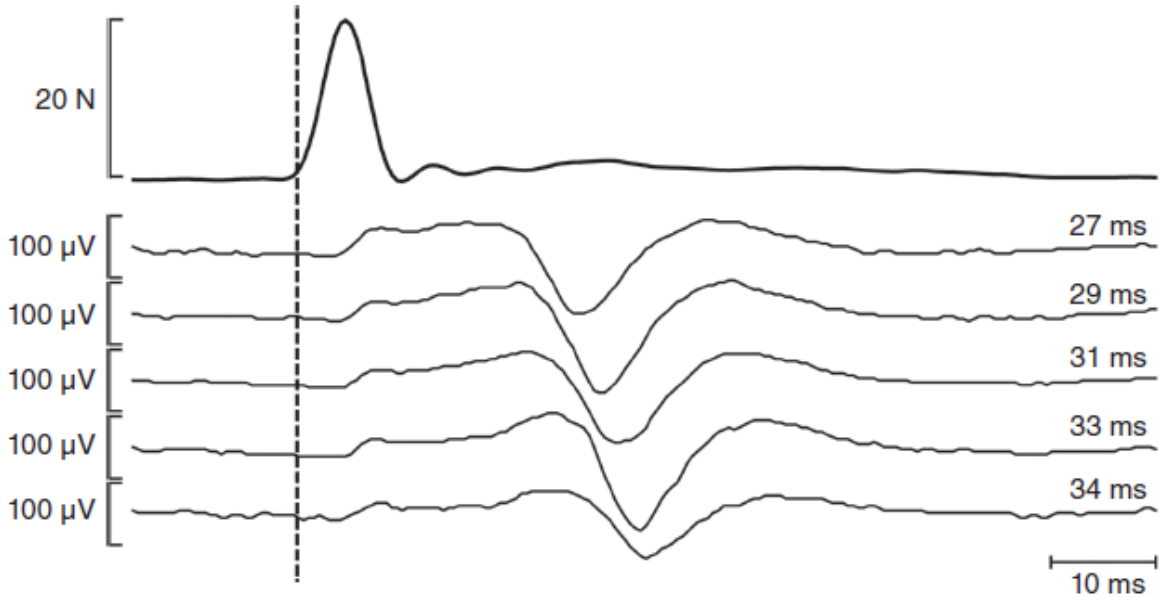


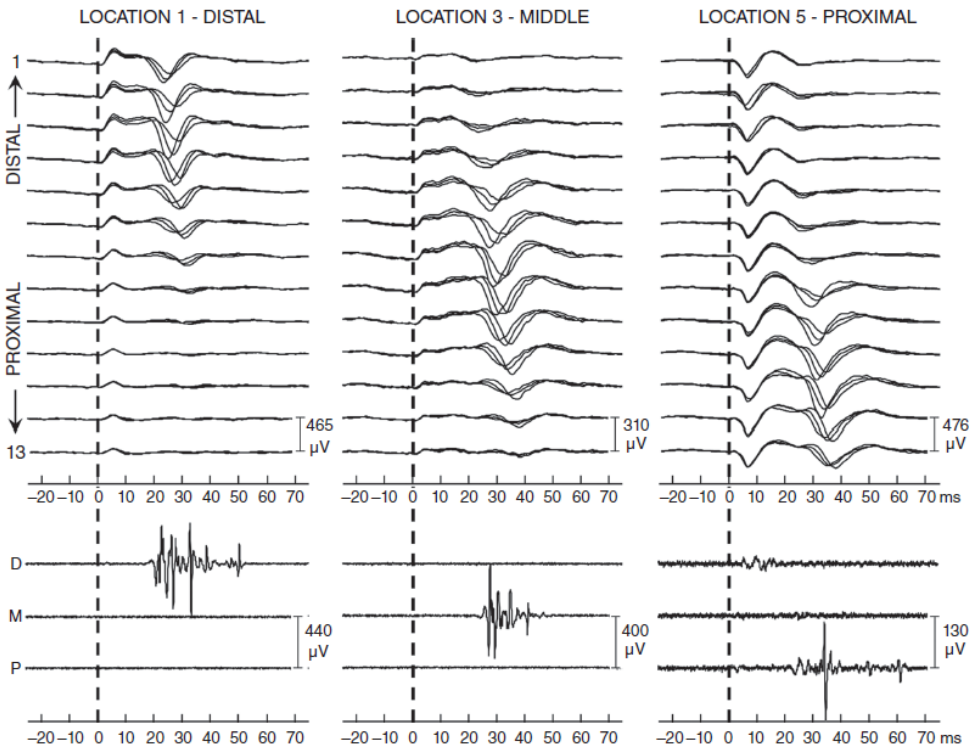
Fig.1: Experimental setup. The innervation zone is illustrated as a dashed black line. Black crosses identify the average location of the tendon taps (mean and standard deviation) across participants. The surface EMG amplitude plot on top of the electrode grid represents the expected spatial distribution of the response to the tap of location 4. The target location for the intramuscular wires is indicated by the three black diamonds.



476

477 Fig.2: Identification of a surface EMG response. The force signal used to determine the tap onset is
478 depicted on top. EMG signals from five channels along the muscle fibre orientation are plotted in the
479 channels below; numbers on the right side of the plot are latency estimates of the negative peak of the
480 action potential. Physiological action potential propagation latencies ensured the distinction of artefacts
481 from EMG reflexes.

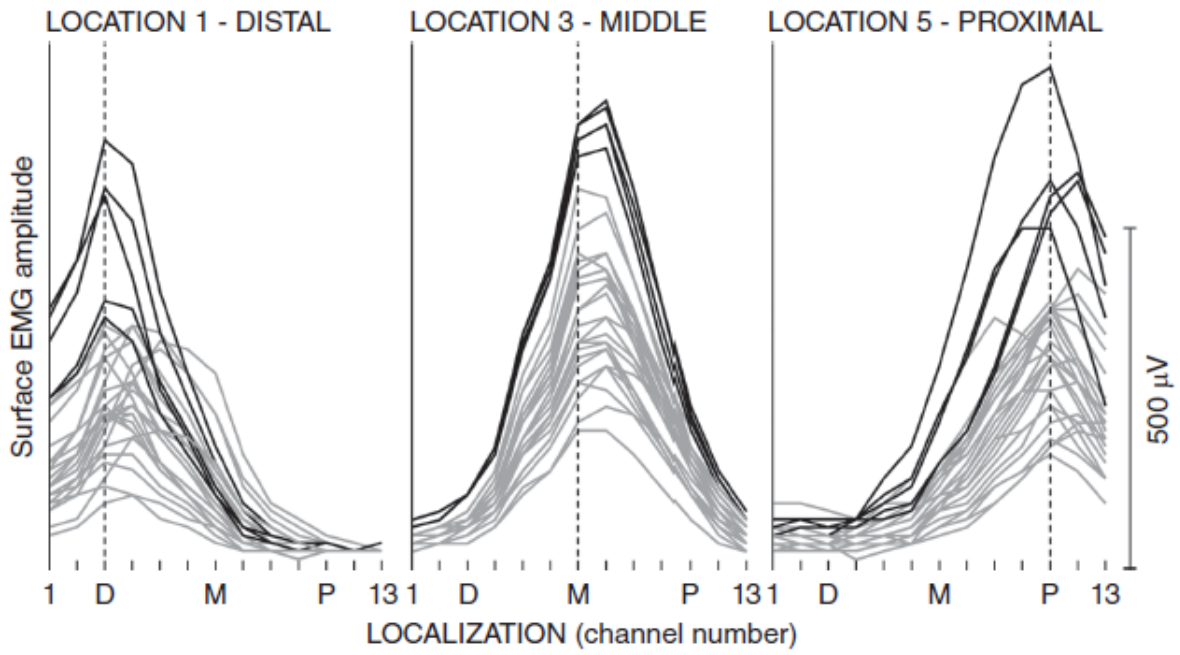
482



483

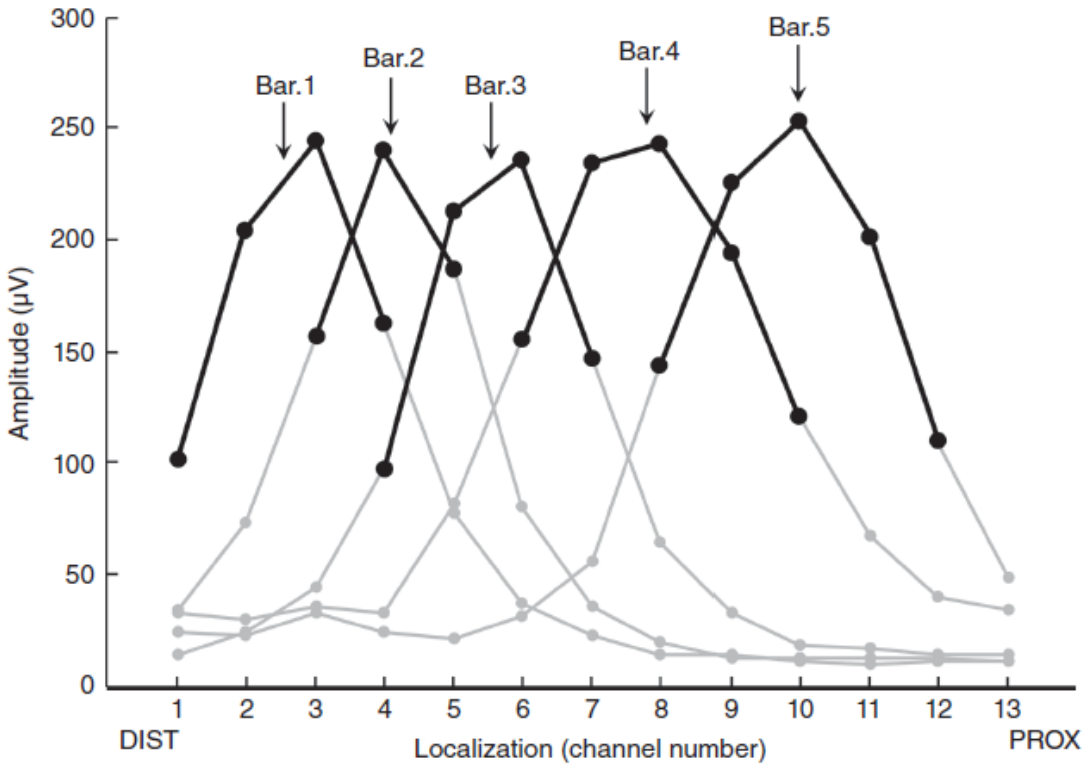
484 Fig.3: Example of responses to taps of location 1 (left panels), 3 (middle panels), and 5 (right panels) for
485 participant 3 (Table 1). Surface EMGs channels (top panels) are organized from distal (ch.1) to proximal
486 (ch.13); each row shows EMG signals from three channels placed along the approximate fibre orientation.
487 For intramuscular EMG signals (bottom panels), the top signals was collected from the wire inserted in
488 the distal region of the VM, the bottom ones from the most proximal.

489



490

491 Fig.4: Responses to individual mechanical taps in three locations for a representative participant. The
492 location of the proximal (P), middle (M) and distal (D) intramuscular wires are depicted. Black lines
493 identify the five responses with highest amplitude; for each location, the spatial location of the response
494 is similar across taps. The location of the intramuscular wire in relation to the HDsEMG grid is presented
495 with dashed line in each panel.



496

497 Fig.5: Identification of size and location of EMG responses across the grid. Each gray line is the amplitude

498 distribution calculated from five responses, matched for amplitude across locations. The arrows identify

499 the barycentre (Bar) of the channels above the threshold (black circles) for taps of locations 1 to 5.

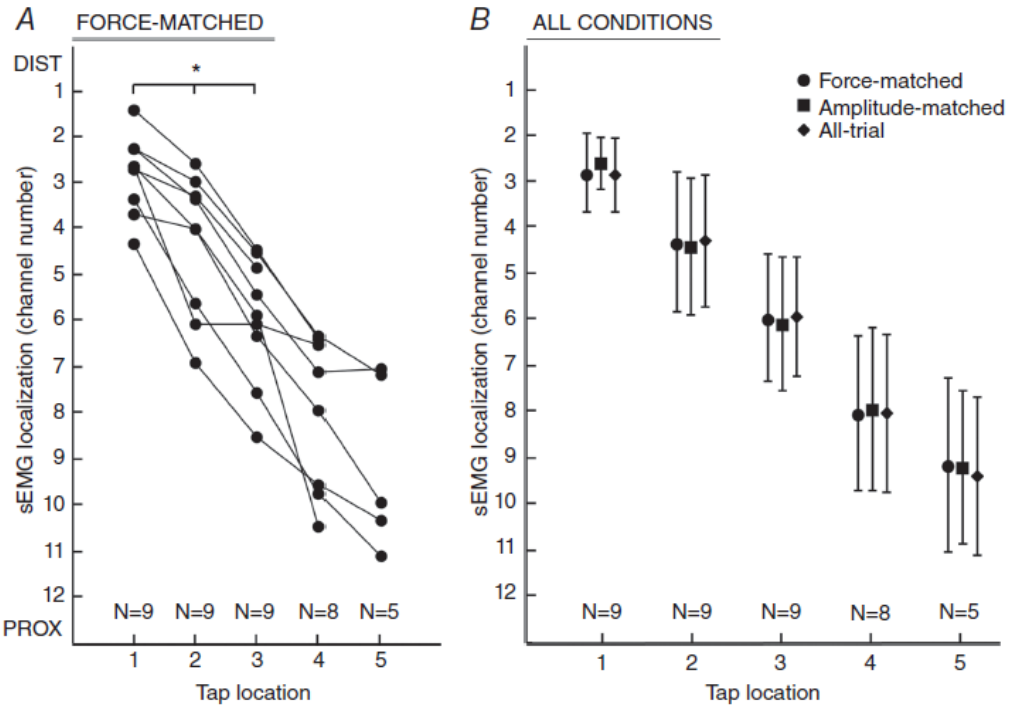


Fig.6: A) Effect of tap location on the localization of the response, force-matched condition. Lines depict the position of the responses on the grid for individual participants. * $P < 0.001$. B) Localization of the responses for force-matched, amplitude-matched and all-trial conditions.